

IFWO

RAW SEQUENCE LISTING

DATE: 08/31/2004

PATENT APPLICATION: US/10/757,909

/10/757.909 TIME: 13:36:49

```
1 <110> APPLICANT: DeBonte, Lorin R.
              Fan, Zhegong
              Miao, Guo-Hua
      ٦
      4 <120> TITLE OF INVENTION: FATTY ACID DESATURASES AND MUTANT SEQUENCES THEREOF
      5 <130> FILE REFERENCE: 07148-063003
      6 <140> CURRENT APPLICATION NUMBER: US/10/757,909
      7 <141> CURRENT FILING DATE: 2004-01-15
      8 <150> PRIOR APPLICATION NUMBER: US/09/771,904
      9 <151> PRIOR FILING DATE: 2001-01-29
    10 <150> PRIOR APPLICATION NUMBER: US 08/874,109
    11 <151> PRIOR FILING DATE: 1997-06-12
    12 <160> NUMBER OF SEQ ID NOS: 70
    13 <170> SOFTWARE: FastSEQ for Windows Version 4.0
                                                           A CHANGE A PROPERTY OF A TO A SECOND
    15 <210> SEO ID NO: 1
    16 <211> LENGTH: 1155
    17 <212> TYPE: DNA
    18 <213> ORGANISM: Brassica napus
    19 <220> FEATURE:
    20 <221> NAME/KEY: CDS
    21 <222> LOCATION: (1)...(1152)
    22 <223> OTHER INFORMATION: Wild type Fad2
    23 <220> FEATURE:
    24 <221> NAME/KEY: misc feature
    25 <222> LOCATION: 205
    \cdot26 <223> OTHER INFORMATION: n = a, g, c, or t/u
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                                                                                       48
    28
              Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser
    29
    3.0
                                                    10
                                                                                       96
    31
              gaa acc gac acc atc aag cgc gta ccc tgc gag aca ccg ccc ttc act
              Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
    32
                                                25
    33
              gto gga gaa oto aag aaa goa ato ooa oog cao tgt tto aaa ogo tog
                                                                                      144
    34
              Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
    35
    36
                                            40
    37
              ate cet ege tet the tee tae etc ate tgg gae ate ate ata gee tee
                                                                                      192
              Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
    38
    39
                                        55
              tgc ttc tac tac ntc gcc acc act tac ttc cct ctc ctc cct cac cct
                                                                                      240
W - - > 40
              Cys Phe Tyr Tyr Xaa Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
 --> 41
    42
                                    70
                                                        75
              ete tee tae tte gee tgg eet ete tae tgg gee tge eaa ggg tge gte
                                                                                      288
    43
              Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
```

RAW SEQUENCE LISTING DATE: 08/31/2004 PATENT APPLICATION: US/10/757,909 TIME: 13:36:49

											•							
45					85					90					95			
46	cta	acc	ggc	gtc	tgg	gtc	ata	gcc	cac	gaa	tgc	ggc	cac	cac	gcc	ttc		336
47	Leu	Thr	Gly	Val	Trp	Val	Ile	Ala	His	Glu	Cys	Gly	His	His	Ala	Phe		
48				100					105					110				
4 9	agc	gac	tac	cag	tgg	ctt	gac	gac	acc	gtc	ggt	ctc	atc	ttc	cac	tcc		384
50	Ser	Asp	Tyr	Gln	Trp	Leu	Asp	Asp	Thr	Val	Gly	Leu	Ile	Phe	His	Ser		
51			115					120					125					
52	ttc	ctc	ctc	gtc	cct	tac	ttc	tcc	tgg	aag	tac	agt	cat	cgc	agc	cac		432
53	Phe	Leu	Leu	Val	Pro	Tyr	Phe	Ser	Trp	Lys	Tyr	Ser	His	Arg	Ser	His		
54		130					135					140						
55	cat	tcc	aac	act	ggc	tcc	ctc	gag	aga	gac	gaa	gtg	ttt	gtc	CCC	aag		480
56	His	Ser	Asn	Thr	Gly	Ser	Leu	Glu	Arg	Asp	Glu	Val	Phe	Val	Pro	Lys		
57	145					150					155					160		
58	aag	aag	tca	gac	atc	aag	tgg	tac	ggc	aag	tac	ctc	aac	aac	cct	ttg		528
59	Lys	Lys	Ser	Asp	Ile	Lys	Trp	Tyr	Gly	Lys	Tyr	Leu	Asn	Asn	Pro	Leu		
60	-				165	_	-			170	,				175			
61	gga	cgc	acc	gtg	atg	tta	acg	gtt	çag	ttc	act	ctc	ggc	tgg	ccg	ttg		576
62	Gly	Arg	Thr	Val	Met	Leu	Thr	Val	Gln	Phe	Thr	Leu	Gly	Trp	Pro	Leu		
63 .				180					185					190				
64	tac	tta	gcc	ttc	aac	gtc	tcg	gga	aga	cct	tac	gac	ggc	ggc	ttc	cgt		624
65 <i>'</i>					Asn													
66			195					200					205					
67	tgc	cat	ttc	cac	CCC	aac	gct	CCC	atc	tac	aac	gac	cgc	gag	cgt	ctc		672
68	Cys	His	Phe	His	Pro	Asn	Ala	Pro	Ile	Tyr	Asn	Asp	Arg	Glu	Arg	Leu	-	
69		210					215					220						
70	cag	ata	tac	atc	tcc	gac	gct	ggc	atc	ctc	gcc	gtc	tgc	tac	ggt	ctc		720
71	Gln	Ile	Tyr	Ile	Ser	Asp	Ala	Gly	Ile	Leu	Ala	Val	Cys	Tyr	Gly	Leu		•
72 .	225					230					235					240		
73	ttc	cgt	tac	gcc	gcc	ggc	cag	gga	gtg	gcc	tcg	atg	gtc	tgc	ttc	tac		768
74	Phe	Arg	Tyr	Ala	Ala	Gly	${\tt Gln}$	Gly	Val	Ala	Ser	Met	Val	Cys	Phe	Tyr		
75					245					250					255			
76					ctg													816
77	Gly	V al	Pro	Leu	Leu	Ile	Val	Asn	Gly	Phe	Leu	Val	Leu	Ile	Thr	Tyr		
78				260					265					270				
79	_	_		_	cat			_				-	_					864
80	Leu	Gln	His	Thr	His	Pro	Ser	Leu	Pro	His	Tyr	Asp	Ser	Ser	Glu	Trp		
81			275					280					285					
82	gat	tgg	ttc	agg	gga	gct	ttg	gct	acc	gtt	gac	aga	gac	tac	gga	atc		912
83	Asp	${\tt Trp}$	Phe		Gly									Tyr	Gly	Ile		
84		290					295					300						
85	ttg	aac	aag	gtc	ttc	cac	aat	att	acc	gac	acg	cac	gtg	gcc	cat	cat		960
86	Leu	Asn	Lys	Val	Phe	His	Asn	Ile	Thr	Asp	Thr	His	Val	Ala	His	His		
87	305					310					315					320		
88	ccg	ttc	tcc	acg	atg	ccg	cat	tat	cac	gcg	atg	gaa	gct	acc	aag	gcg	1	1008
89	Pro	Phe	Ser	Thr	Met	Pro	His	Tyr	His	Ala	Met	Glu	Ala	Thr	Lys	Ala		
90					325					330					335			
91	ata	aag	ccg	ata	ctg	gga	gag	tat	tat	cag	ttc	gat	ggg	acg	ccg	gtg	1	L056
92	Ile	Lys	Pro	Ile	Leu	Gly	Glu	Tyr	Tyr	Gln	Phe	Asp	Gly	Thr	Pro	Val		
93				340					345					350				

RAW SEQUENCE LISTING DATE: 08/31/2004
PATENT APPLICATION: US/10/757,909 TIME: 13:36:49

	94														tat ·				-	1104
	95	7	Val :	Lys A	Ala N	Met '	$\operatorname{\mathtt{Trp}}$	Arg	Glu .	Ala	Lys	Glu	Cys .	Ile	Tyr	Val (Glu I	Pro		
	96				355					360					365					
	97		_												aac				-	1152
	98		Asp .	Arg (Gln (Gly (Glu	Lys	Lys	Gly	Val	Phe	Trp	_	Asn .	Asn l	Lys 1	Leu		
	99			370					375					380						
	100		tga																	1155
	102	<210>	SEQ	ID I	NO: 2	2														
		<211>																		
		<212>																		
		<213>				rass	ica	napu	S				,							
		<220>								_										
		<223>				MATI	ON:	Xaa	=, Ph	e, L	eu,	He,	or	Val						
		<400>						_				_	_	_	_	_	_	_		
	109				Ala	GLY	GIĀ	Arg	Met	GIn	Val			Pro	Ser	Lys		Ser		
	110		1			m)	5	T	3	**- 7		10		. ml	. D	D	15	ml		
	111		GIu	Thr	Asp		тте	Lys	Arg	vai			GIU	ı Tnr	Pro		Pne	Thr		
	112		•• •	~ 1	~1	20	Ŧ	T	77.	*1 -	25		772		Dlas	30	7	0		
	113		Val	GIY		ьeu	ьуs	Lys	Ата			Pro	HIS	сув	Phe		Arg	ser		
	114		~1.	D	35	a	D1	a		40		Ш	. 7	T 7 A	45		7.7.	0		
	115		ше		Arg	ser	Pne	Ser	_		ıııe	rrp	Asp		lle	шe	Ата	ser		
	116	•	_	50	_	_			55			. DL -		60		D	TT -	D		
•>	117		_		ıyr	туг	хаа			Thr	Tyr	Pne			Leu	Pro	HIS			
	118		65		TT- rac	Dho	77.	70		Τ 011	The same	Trn	75 - רג		Cln	C1.,	Cva	08 1 - V		
	119		Leu	ser	тут	Pile	85		PIO	ьeu	ııyı	90		Сув	Gln	GIY	95	vai		
	120		T 011	The	dī.	77-7			710	7.7.	uia			Cla	His	Uic		Dhe		
	121		ьеи	1111	GIA	100	пр	val	TIE	Ата	105		Сув	, Сту	піз	110	Ата	FIIC		
	122 123		Cor	Λcn	Тъгъ		Trn	LOU	λen	Λαn			G1v	r T.011	ılle		Нic	Ser		
	124		per	дам	115	GIII	тър	шец	. Asp	120		val	. Gry	псс	125		пты	DCI		
	125		Dhe	T.011		Wa 1	Pro	Tur	Dhe			T.vg	Tvr	Ser	His		Ser	His		
	126		TIIC	130	шси	Vai	110	1 7 1	135		111	, шуг	,	140		*** 9	001	1110		
	127	-	His		Asn	Thr	Glv	Ser			Aro	Asn	G]11		Phe	Val	Pro	Lvs		
	128		145		11011		011	150		010		1101	155					160		
	129				Ser	Asp	Tle			Tvr	Glv	Lvs			. Asn	Asn	Pro			
	130		215	275	201	1101	165	-		-1-	0-1	170					175			
	131		Glv	Ara	Thr	val			Thr	Val	Gln			Leu	Glv	Trp		Leu		
	132		U -1			180					185				1	190				
	133		Tvr	Leu	Ala		Asn	Val	Ser	Glv			Tvr	Asc	Gly		Phe	Arq		
	134	•	-1-		195					200			-1-		205			5		
	135		Cvs	His		His	Pro	Asn	Ala			Tvr	Asn	Ast	Arg		Arg	Leu		
	136		-1	210					215			- 1		220			,]			
	137		Gln		Tvr	Ile	Ser	Asp			· Ile	Leu	ı Ala	Val	Cys	Tvr	Gly	Leu		
	138		225		-1-	_ =, _		230		1			235		1 ~	4	- 1	240		
	139				Tvr	Ala	Ala			Glv	Val	Ala			. Val	Cvs	Phe			
	140			9	- , -		245			1		250					255			
	141		Glv	Val	Pro	Leu			Val	Asn	Glv			ı Val	Leu	Ile		Tyr		
	142		1			260					265					270		4		
	143		Leu	Gln	His			Pro	Ser	Leu			Tvr	Asr	Ser		Glu	Trp		
								~										_		

RAW SEQUENCE LISTING DATE: 08/31/2004 PATENT APPLICATION: US/10/757,909 TIME: 13:36:49

	144				275					280					285				
	145		asA	Trp	Phe	Arq	Gly	Ala	Leu	Ala	Thr	Val	Asp	Ara	Asp	Tvr	Glv	Ile	
	146			290		J			295					300		1	-		
	147		Leu		Lvs	Val	Phe	His		Tle	Thr	Asp	Thr		Val	Ala	His	His	
	148		305		-1-			310					315					320	
	149			Phe	Ser	Thr	Met		His	Туг	His	Δla		Glu	Δla	Thr	Lvs		
	150		110	LIIC	DCI	1111	325	110	III	- y -	1111	330	IIC C	OIU	ALU	1111	335	niu	
			тЪо	Tara	Dro	Tla		Cl v	Cl.	Тиг	Тиг		Dho	Λαn	Clar	Thr		Tra I	
	151		тте	пув	PIO		Leu	Gry	GIU	ıyı	345	GIII	Pile	Asp	СТУ		PIO	vaı	
	152		77-7	T	7.7.	340	m	7	a1	77.		a1	G	т1 -	III	350	~1	D	
	153		vai	гуѕ		мęс	Trp	Arg	GIU		ьўѕ	GIU	Cys	ше	_	vai	GIU	PIO	
	154		_	_	355	~-7	~ 7	_	_	360		_1	_		365	_	_	_	•
	155		Asp	_	GIn	GIY	Glu	rys	_	GIY	Val	Phe	Trp	_	Asn	Asn	Lys	Leu	
	156			370					375					380					
		<210>																	
	159	<211>	LENC	GTH:	1155	5													
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	162	<220>	FEAT	FURE:	:														
	163	<221>	NAME	E/KE	Y: CI	DS													
	164	<222>	LOCE	OITA	N: (1)	. (119	52)											
	165	<223>	OTHE	ER I	NFORM	MATIO	ON: (3 to	A tı	rans	vers:	ion r	nutat	ion	at r	nucle	eotic	de 316	
	166	<220>	FEAT	TURE:	:														
	167	<221>	NAME	E/KE	Y: m:	isc :	featı	ıre											
	168	<222>	LOCA	OITA	N: 20	05													
	169	<223>	OTHE	ER II	NFORM	MATI	ON: I	1 = a	a, q	, с,	or t	t/u							
		<400>							. 5.			•							
	171					aat	gga	aga	atq	caa	ata	tct	cct	CCC	tcc	aaq	aaq	tct	48
	172		-		-		Gly										_		
	173		1	1		2	5	5				10				-2-	15		
	174			acc	gac	acc	atc	aaσ	cac	αta	ccc		gag	aca	cca	ccc		act	96
	175						Ile												, ,
	176		O.L.	1111	1101	20	110	цу	111.9	vai	25	o y o	014		110	30			
	177		ata	aaa	gaa		aaq	222	aca	ato		cca	cac	tat	ttc		cac	tea	144
	178		_		_		Lys		_			_		_			_	_	111
	179		vai	Gry	35	пси	пур	цуз	AIG	40	IIO	110	1113	СуБ	45	цуз	Arg	DCI	
	180		2+4	aat		+ 0+	ttc	+ ~ ~	+ = ~		a+~	+~~	a 2a	at~		2+2	aaa	tac	192
					_								_				_		192
	181		тте		Arg	ser	Phe	ser	_	ьец	тте	ıτb	Asp		тте	тте	AId	ser.	
	182			50					55					60					
	183						ntc												240
>	184			Phe	Tyr	Tyr	Xaa		Thr	Thr	Tyr	Phe		Leu	Leu	Pro	Hls		
	185		65					70					75					80	
	186						gcc												288
	187		Leu	Ser	Tyr	Phe	Ala	Trp	Pro	Leu	Tyr		Ala	Cys	Gln	Gly	_	Val	
	188						85					90					95		
	189						tgg												336
	190		Leu	Thr	Gly	Val	${\tt Trp}$	Val	Ile	Ala	His	Lys	Cys	Gly	His	His	Ala	Phe	
	191					100					105					110			
							+~~	a++	~~~	~~~							000		204
	192		agc	gac	Lac	cag	ugg	CLL	gac	gac	acc	gtc	ggt	CLC	atc	tte	Cac	Lec	384
	192 193						Trp												384

RAW SEQUENCE LISTING

DATE: 08/31/2004 PATENT APPLICATION: US/10/757,909 TIME: 13:36:49

194			115					120					105					
195	ttc	ctc		ata	aat	tan	ttc		+~~	224	+	204	125	000	200	a2a		422
196							Phe											432
197	FIIC	130	пец	vai	FIO	тут		ser	пр	цув	TAT		HIS	Arg	ser	HIS		
							135					140						
198							ctc											480
199		ser	Asn	Thr	GIA		Leu	GIU	Arg	Asp		Val	Phe	Val	Pro	-		
200	145					150					155					160		
201							tgg											528
202	Lys	Lys	Ser	Asp		Lys	Trp	Tyr	Gly		\mathtt{Tyr}	Leu	Asn	Asn		Leu		
203					165					170				•	175			
204							acg											576
205	Gly	Arg	Thr		Met	Leu	Thr	Val	Gln	Phe	Thr	Leu	Gly	Trp	Pro	Leu		
206				180					185					190				
207							tcg											624
208	Tyr	Leu	Ala	Phe	Asn	Val	Ser	Gly	Arg	Pro	Tyr	Asp	Gly	Gly	Phe	Arg		
209			195					200					205					
210							gct											672
211	Cys	His	Phe	His	Pro	Asn	Ala	Pro	Ile	Tyr	Asn	Asp	Arg	Glu	Arg	Leu		
212		210					215					220						
213	cag	ata	tac	atc	tcc	gac	gct	ggc	atc	ctc	gcc	gtc	tgc	tac	ggt	ctc		720
214	Gln	Ile	Tyr	Ile	Ser	Asp	Ala	Gly	Ile	Leu	Ala	Val	Cys	Tyr	Gly	Leu		
215	225					230					235					240		
216	ttc	cgt	tac	gcc	gcc	ggc	cag	gga	gtg	gcc	tcg	atg	gtc	tgc	ttc	tac		768
217							${\tt Gln}$											
218 .					245					250				_	255	_		
219	gga	gtc	ccg	ctt	ctg	att	gtc	aat	ggt	ttc	ctc	gtg	ttg	atc	act	tac		816
220							Val											
221	_			260					265					270		•		
222	ttg	cag	cac	acq	cat	cct	tcc	ctq	cct	cac	tac	qat	tcq	tcc	gag	taa		864
223							Ser											
224			275					280			-	-	285			-		
225	qat	tgg	ttc	agg	qqa	qct	ttg	qct	acc	att	qac	aga	gac	tac	gga	atc		912
226							Leu											
227	_	290		_	-		295				-	300	-	4	4			
228	ttg	aac	aaq	gtc	ttc	cac	aat	att	acc	qac	acq	cac	ata	qcc	cat	cat		960
229							Asn											
230	305		-			310				_	315					320		
231	ccq	ttc	tcc	acq	atq	cca	cat	tat	cac	aca	atq	gaa	act	acc	aaq		1	1008
232							His											
233					325										335			
234	ata	aaq	cca	ata			gag					gat	aaa				1	.056
235							Glu											.050
236		_1		340		1		-1-	345					350				
237	at.t.	aag	ara		taa	agg	gag	aca		nan	tat	atc	tat		caa	cca	1	.104
238							Glu										4	
239		-15	355	-100	P	9	JIU	360	y	JIU	Cys	110	365	vai	Jiu	110		
240	asc	add		aat	nan	224	aaa		ata	tta	taa	tac		aat	224	++=	. 1	.152
241							Lys										1	. I D Z
242	Tob	370	GIII	GT Å	JIU	пур		дт У	val	FIIG	ттр		ASII	ASII	пув	π e π		
~ * ~		3/0					375					380						

RAW SEQUENCE LISTING ERROR SUMMARY

DATE: 08/31/2004

PATENT APPLICATION: US/10/757,909

TIME: 13:36:50

Input Set : N:\Crf3\RULE60\10757909.raw.txt Output Set: N:\CRF4\08312004\J757909.raw

Please Note:

Use of n and/or Xaa have been detected in the Sequence Listing. Please review the Sequence Listing to ensure that a corresponding explanation is presented in the <220> to <223> fields of each sequence which presents at least one n or Xaa.

Seq#:1; N Pos. 205

Seq#:1; Xaa Pos. 69

Seq#:2; Xaa Pos. 69

Seq#:3; N Pos. 205

Seq#:3; Xaa Pos. 69

Seq#:4; Xaa Pos. 69

VARIABLE LOCATION SUMMARY

DATE: 08/31/2004

PATENT APPLICATION: US/10/757,909

TIME: 13:36:50

Input Set : N:\Crf3\RULE60\10757909.raw.txt
Output Set: N:\CRF4\08312004\J757909.raw

Use of n's or Xaa's (NEW RULES):

Use of n's and/or Xaa's have been detected in the Sequence Listing. Use of <220> to <223> is MANDATORY if n's or Xaa's are present. in <220> to <223> section, please explain location of n or Xaa, and which residue n or Xaa represents.

Seq#:1; N Pos. 205

Seq#:1; Xaa Pos. 69

Seq#:2; Xaa Pos. 69

Seq#:3; N Pos. 205

Seq#:3; Xaa Pos. 69

Seq#:4; Xaa Pos. 69

VERIFICATION SUMMARY

DATE: 08/31/2004 TIME: 13:36:50

PATENT APPLICATION: US/10/757,909

TENT APPLICATION: 05/10//5/,909

Input Set : N:\Crf3\RULE60\10757909.raw.txt
Output Set: N:\CRF4\08312004\J757909.raw

L:40 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:1 after pos.:192
L:41 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:1 after pos.:240
L:117 M:258 W: Mandatory Feature missing, <221> Tag not found for SEQ ID#:2
L:117 M:258 W: Mandatory Feature missing, <222> Tag not found for SEQ ID#:2
L:117 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:2 after pos.:64
L:183 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:3 after pos.:192
L:184 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:3 after pos.:240
L:260 M:258 W: Mandatory Feature missing, <221> Tag not found for SEQ ID#:4
L:260 M:258 W: Mandatory Feature missing, <222> Tag not found for SEQ ID#:4
L:260 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:4 after pos.:64